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ORIGINAL PAPER

Identification of QTLs for resistance to powdery mildew and SSR markers diagnostic for powdery mildew resistance genes in melon (*Cucumis melo* L.)

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Abstract Powdery mildew caused by *Podosphaera xanthii* is an important foliar disease in melon. To find molecular markers for marker-assisted selection, we constructed a genetic linkage map of melon based on a population of 93 recombinant inbred lines derived from crosses between highly resistant AR 5 and susceptible 'Earl's Favourite (Harukei 3)'. The map spans 877 cM and consists of 167 markers, comprising 157 simple sequence repeats (SSRs), 7 sequence characterized amplified region/cleavage amplified polymorphic sequence markers and 3 phenotypic markers segregating into 20 linkage groups. Among them, 37 SSRs and 6 other markers were common to previous maps. Quantitative trait locus (QTL) analysis identified two loci for resistance to powdery mildew. The effects of these QTLs varied depending on strain and plant stage. The percentage of phenotypic variance explained for resistance to the pxA strain was similar between QTLs (R^2 = 22–28%). For resistance to pxB strain, the QTL on linkage group (LG) XII was responsible for much more of the variance (41–46%) than that on LG IIA (12–13%). The QTL on LG IIA was located between two SSR markers. Using an independent population, we demonstrated the effectiveness of these markers. This is the first report of universal and effective markers linked to a gene for powdery mildew resistance in melon.

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Introduction

Powdery mildew caused by *Podosphaera xanthii* (syn. *Sphaerotheca fuliginea* ex Fr. Poll.) and, to a lesser degree, *Golovinomyces cichoracearum* (syn. *Erysiphe cichoracearum* DC ex Mérat.) limits the production of melon (*Cucumis melo* L.) worldwide (Sitterly 1978). Many races of powdery mildew have been identified, and resistant cultivars or accessions have been reported (Sowell and Corley 1974; Epinat et al. 1993; Alvarez et al. 2005; McCreight et al. 2005). Recently, the appearance of new races of these fungi and the breakdown of disease resistance have been reported (Hosoya et al. 2000; McCreight et al. 2005). Xu et al. (2006) proposed that methods for breeding durable powdery-mildew-resistant wheat cultivars must include pyramiding of race-specific genes, the use of partial-resistance genes, or a combination of both. If molecular markers tightly linked to the target genes are available, pyramiding resistance genes by marker-assisted selection (MAS) may speed the development of resistant cultivars. In melon, several resistance genes have been mapped or cloned: for resistance to *Fusarium* wilt (*Fom-1*; Brotman et al. 2005, *Fom-2*; Baudracco-Arnas and Pitrat 1996; Joobeur et al. 2004), papaya ring spot virus (*Prv*; Brotman et al. 2005), zucchini yellow mosaic virus (*Zym*; Danin-Poleg et al. 2002), Virus aphid transmission (*Vat*; Pitrat and Lecoq 1980), and melon necrotic spot virus (MNSV) (*nsv*; Morales et al. 2005, Nieto et al. 2006). Several genes and QTLs for resistance to powdery mildew have been mapped: *Pm-w* of WMR 29, *Pm-x* of PI 414723 and *Pm-y* of VA 435 (Pitrat 1991; Périn et al. 2002), *PmV.1* and *PmXII.1* of PI 124112 (Perchepied

et al. 2005). However, there is no publicly available, tightly linked, universal molecular marker for any of these genes. One reason may be the difficulties in analysing powdery mildew resistance due to the influence of environmental factors on the development of the disease. One possible solution to this issue is the analysis of recombinant inbred lines (RILs) in replicated experiments in different environments (Paran et al. 1995).

Simple sequence repeats (SSRs) are highly polymorphic, codominant, transferable molecular markers that are suitable for MAS and as anchor points for merging linkage maps. SSRs can be used to build consensus maps that integrate information on markers segregating in different crosses and provide an important framework for producing and exchanging genetic information (Wenzl et al. 2006). Several studies have used melon SSRs in map comparison (Danin-Poleg et al. 2000; Silberstein et al. 2003; Gonzalo et al. 2005; Zalapa et al. 2007). For comparison, we used the Ved_PI (Périn et al. 2002), PI_PS (Gonzalo et al. 2005), and US_TM (Zalapa et al. 2007) maps.

AR 5 (previously referred as PMAR No. 5 by Yoshida and Iwanaga 1991 and Fukino et al. 2004) is a cantaloupe breeding line that was introduced into Japan from the University of California in 1981 (Yoshida and Kohyama 1986). AR 5 has cotton-melon aphid (*Aphis Gossypii*) resistance derived from Indian accession PI 371795. AR 5 is nearly isogenic to the aphid-susceptible breeding line PMR 5, as its pedigree includes eight successive backcrosses to PMR 5 (McCreight et al. 1984). Therefore, its resistance to powdery mildew is thought to be the same as that of PMR 5. PMR 5 has resistance to powdery mildew races 0, 1, 2 Fr, 2 US, 4 and 5 (Bardin et al. 1999), as well as to all four new races identified by Hosoya et al. (2000). AR 5 and PMR 5 have been used as powdery-mildew-resistant parents, but few reports address the genetics of the powdery mildew resistance of AR 5 (Fukino et al. 2004) and PMR 5 (Epinat et al. 1993; Floris and Alvarez 1995). Furthermore, there have been few efforts to elucidate the interaction between the resistant genes and the fungal strains.

We report here the construction of a genetic linkage map through the use of SSR, sequence characterized amplified region (SCAR) and cleavage amplified polymorphic sequence (CAPS) markers and agronomically important phenotypic traits (MNSV resistance, *A. gossypii* tolerance and flesh colour) derived from RILs from a cross between AR 5 and 'Earl's Favourite (Harukei 3)' (hereinafter Harukei 3), a susceptible Japanese cultivar. We compare the linkage map with previous maps to test the transferability of the SSR markers. In addition, we used the map to detect quantitative trait loci (QTL) for powdery mildew resistance, using two strains which differ in pathogenicity, to find SSR markers linked to powdery mildew resistance for their use in MAS or diagnosis.

Materials and methods

Plant materials and DNA extraction

The population of 93 RILs (F_8–F_{12}) was derived by single-seed descent from reciprocal crosses between AR 5 and Harukei 3. AR 5 is highly resistant to powdery mildew, MNSV and *A. gossypii*. Its fruit flesh is orange. Harukei 3 is susceptible to those diseases and pest, and its fruit flesh is green. Genomic DNA was isolated from young leaves of the parents and their RILs with a DNeasy Plant Mini Kit (Qiagen, Tokyo, Japan), and used for marker analysis.

Powdery mildew strains

Powdery mildew fungi were collected at the Plant Biotechnology Institute, Ibaraki Agricultural Center, Japan. We tested the responses of seven differential genotypes (listed in Table 1) at two plant stages (cotyledon and first leaves) to two isolates (denoted pxA and pxB) of *P. Xanthii*, each derived from a single ascospore. Two leaf discs (diameter, 15 mm) were cut from two different plants of each genotype and 14 discs from 7 genotypes were placed adaxial side up in a Petri dishes (diameter, 9 cm; agar concentration, 8 g/L). Samples were inoculated in a sedimentation tower as described by Fukino et al. (2004). The Petri dishes were then covered and incubated at 25°C under a 16-h photoperiod. After 10–14 days, the degree of sporulation was scored by eye on a scale of 0 (no sporulation) to 5 (entire disc covered with heavy sporulation) as a disease index (DI). The mean DI from the two independent tests of each genotype in response to the two isolates is shown in Table 1. The responses to both isolates were not significantly different (Wilcoxon *t*-test with Bonferroni correction, $P > 0.05$) between the cotyledon and first-leaf stages. Powdery mildew race can be assigned by comparing reactions of different genotypes at true-leaf stage (Bardin et al. 1999, Hosoya et al. 1999). According to the results in Table 1 for the first leaf, strains pxA and pxB were race N1 (Hosoya et al. 2000) and race 1 (Bardin et al. 1999), respectively.

Powdery mildew resistance test

Resistance to powdery mildew in the two parental lines, F_1 plants and 93 RILs at two plant stages was tested by using the two isolates listed above. Three leaf discs per line were cut from three different plants. Nine discs from three lines and three discs from two parents and their F_1 plant were placed in a petri dish. Three to 26 dishes were inoculated at the same time. Samples were inoculated with powdery mildew by placing them in a sedimentation tower (Fukino et al. 2004) or by spraying them with a conidial suspension

Table 1 Mean (standard deviation) of disease index [scored by eye on a scale of zero (no sporulation) to five (entire disk covered with heavy sporulation)] after inoculation of various genotypes of melon with two strains of *Podosphaera xanthii* (pxA, pxB)

Genotype	pxA		pxB	
	Cotyledon	First-leaf	Cotyledon	First-leaf
PMR 45	2.3 (0.5)	1.0 (1.2)	0.3 (0.5)	0.0 (0.0)
WMR 29	1.3 (1.5)	0.5 (1.0)	0.0 (0.0)	0.0 (0.0)
Edisto 47	2.5 (0.6)	1.3 (1.0)	0.0 (0.0)	0.0 (0.0)
PI 414723	5.0 (0.0)	3.3 (0.5)	2.8 (0.5)	0.5 (1.0)
PMR 5	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Harukei 3	5.0 (0.6)	5.0 (0.0)	4.0 (0.0)	4.5 (0.6)
MR 1	3.3 (0.5)	3.5 (0.6)	0.0 (0.0)	0.0 (0.0)

(Morishita et al. 2003). Samples were incubated and scored as above. The total number of RILs varied between 84 and 93 on account of missing data. The average DI from the two independent tests of each line was used for QTL analysis. The differences of DIs between plant stages or strains were assessed by using Wilcoxon *t*-test with Bonferroni correction.

Phenotypic characters

MNSV resistance

MNSV resistance is conferred by a single recessive gene (*nsv*). We tested resistance as described by Sugiyama and Sakata (2004) with eight plants per line. Seeds were sown in a flat and grown under greenhouse conditions. When the cotyledons were fully expanded, they were cut off, placed on wet filter paper in 9-cm-diameter Petri dishes and dusted with carborundum. They were mechanically inoculated with isolates of Japanese MNSV (MNSV-NH) by rubbing with pieces of cotton soaked in inoculum. The Petri dishes were then covered and incubated at 25°C with 16-h photoperiod. Necrotic lesions appeared 7 days after inoculation. Susceptible plants developed necrotic lesions, whereas resistant plants exhibited no symptoms.

A. gossypii tolerance

One dominant gene (allele *Ag*) confers tolerance to *A. gossypii* in PI 414723 (Bohn et al. 1973) and in AR 5 (Fukino et al. 2004). We tested tolerance as described by Fukino et al. (2004).

Flesh colour

Melon flesh colour has been proposed to be controlled by two genes, *wf* and *gf* (Monforte et al. 2004): fruit with wf^+-/gf^+-.and wf^+-/*gfgf* allelic combinations have orange flesh; those with *wfwf*/gf^+- have white flesh; and *wfwf*/*gfgf* have green flesh (Clayberg 1992). Because the flesh colour of the same RILs used in this study segregated as a monogenic trait (Fukino et al. 2004), AR 5 (orange flesh) would have the combination wf^+wf^+/*gfgf* and Harukei 3 (green flesh) *wfwf*/*gfgf*. The flesh colour of each line was evaluated as described by Fukino et al. (2004).

SSR markers

We screened 556 SSRs from melon and cucumber (CMACC, CMXX, CSXX, Danin-Poleg et al. 2001, CSWXX; Fazio et al. 2002, CMMS; Chiba et al. 2003, CMBR; Ritschel et al. 2004, CMXXN, TJ; Gonzalo et al. 2005, CMN; Fukino et al. 2007, ECM-, GCM-; Fernandez-Silva et al. 2008) for polymorphism between parents. Polymorphic markers were used for the genotyping of RILs. PCR amplification was carried out in 10 μL containing 1–5 ng of extracted genomic DNA, 1 μM each primer, 200 μM of dNTPs, 1× reaction buffer (Takara, Shiga, Japan), and 0.5 unit of Taq polymerase (Takara). DNA was amplified in a GeneAmp PCR System 9700 (Applied Biosystems, Tokyo, Japan) under the following protocol: 94°C for 2 min; 25 cycles of 94°C for 1 min, 50°C for 1 min, 72°C for 1 min; and 72°C for 7 min. PCR products were labeled by post-PCR labeling (Rudi et al. 2002) using R110-ddUTP (Perkinelmer, Yokohama, Japan). The sizes of fragments were estimated using an automated DNA analyzer (model 3730*xl* or ABI PRISM 3100, Applied Biosystems).

SCAR and CAPS markers

SCAR and CAPS markers were derived from RFLP probes (Oliver et al. 2001). Some of them were reported by Morales et al. (2004), and others were designed from sequences taken from the MELOGEN database (http://www.melogen.upv.es, Gonzalez-Ibeas et al. 2007). They were amplified from both AR 5 and Harukei 3. The PCR protocol was the same as described in '*SSR markers*'. The PCR products were purified with a Montage PCR96 cleanup kit (Millipore, Tokyo, Japan) and sequenced with an ABI PRISM BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems) on an ABI PRISM 3100 DNA analyser. Sequences obtained from the two parents were aligned with the Genetyx software (Genetyx, Tokyo, Japan). By comparing sequences, we found size differences or predicted some endonucleases that would generate polymorphic fragments and designed internal primers for easy detection of the polymorphisms by the Genetyx software (Table 2). For SCAR markers, PCR and detection were performed as described in '*SSR markers*'. For CAPS markers, protocols for PCR were as described in '*SSR markers*', and

Table 2 SCAR and CAPS markers used in this study

Marker	Sense primer 5′–3′ (top) Antisense primer to 5′–3′ (bottom)	Endonuclease for CAPS	Size of polymorphic fragment (bp)		Reference
			AR 5	Harukei 3	
CM1.15	ATTTCTTTTTCCTAATATTTAAC GAGAACTAATTCGTATGGTTTA		204	202	Morales et al. (2004)
CM1.41	TTCATTTCAGATCTGGCTCTCTG ACAGCCTGAAAGTGAACCT		91	92	Morales et al. (2004)
CM2.20	GATGATGGTTGAAGTTGGAGA TCATCACCAAAATGTTCATTAAGC		146	147	Morales et al. (2004)
MC224	GCTTGCTACTTAACGTTTG GACATGCATAATGTGAGAAG		120	123	Morales et al. (2004)
MC051	TCGTCAACCACCTTATGCAA TTTTGGCCATCTTCTGGAAC	*Bcn*I	64 + 300	364	http://www.melogen.upv.es
MC279	TATCGGCCTTGGTATTCCTG AGGGCCACAGTTTCAATCAC	*Alw*I	329	100 + 229	http://www.melogen.upv.es
MC340	AGGCATACAACTCAGA GCATTCTTCCTACTCCAA	*Mbo*II	101 + 108	209	http://www.melogen.upv.es

restriction endonuclease treatment and detection were performed as described by Kunihisa et al. (2003).

Linkage map construction and QTL detection

The linkage map obtained from the AR-5× Harukei-3 RIL population ("AR5_H3 map") was constructed with MAPMAKER/EXP 3.0 (Lander et al. 1987). Marker data were assigned to linkage groups (LGs) by using a minimum logarithm of odds (LOD)-likelihood score of 5.0 (143 markers) or 3.0 (24 markers). The Kosambi map function (Kosambi 1944) was used to calculate the genetic distance between markers. Ordering of markers was done by order command with a LOD score of 3.0. Three maps (Ved_PI, PI_PS and US_TM) were used for comparison. Among them, the PI_PS map was constructed exclusively with RFLP, SSR and SNP markers and can be used for alignment of LGs by using common SSR, SCAR and CAPS markers. The nomenclature of LGs corresponds to that in Périn et al. (2002). For LGs with no common markers, markers from the AR5_H3 map were mapped on the PI_PS map by selective genotyping using the bin-mapping strategy (Moreno et al. 2008). QTL analysis was performed by using both the Kruskal–Wallis nonparametric rank-sum test (Lehmann 1975) with MapQTL 5 (van Ooijen 2004) and composite interval mapping (CIM) (Zheng 1993, 1994) with Windows QTL Cartographer v2.5 (Wang et al. 2007). The threshold value for assigning a QTL to a map position was $P < 0.001$ by the Kruskal–Wallis test. For CIM, the putative QTL for each trait was estimated from the calculated LOD score after 1000 permutation tests (≥2.8 LOD for resistance to pxA at first-leaf stage, ≥2.9 LOD for others). To test whether the detected QTLs are additive, we grouped RILs according to the genotype of the markers linked to the QTLs, and assessed the differences among genotype groups with the Kruskal–Wallis nonparametric test and the Mann–Whitney *U*-test with Bonferroni's correction.

Validation of marker–resistance association in an independent population

We evaluated a population of 179 F_2 plants derived from a cross between the breeding lines MSL031L-5-26 (susceptible) and M03-6 (a derivative of AR 5, resistant), both developed at NIVTS, for powdery mildew resistance and genotyped plants with resistance-linked markers defined with the AR5_H3 population. Genomic DNA was isolated from cotyledons of the parents and their F_2 plantlets on Multiscreen NA and FB filter plates (Millipore) as described by Matsumoto et al. (2005). Markers linked to resistance QTLs (see "Results") were used for screening of polymorphism between parents and polymorphic markers were used for genotyping of F_2 individuals. The PCR conditions and method of genotyping were as described in '*SSR markers*'. Resistance of F_2 individuals to powdery mildew was evaluated with pxA. Each F_2 was sown in soil on a cell tray in a greenhouse. When cotyledons were fully expanded, they were inoculated by spraying a conidial suspension. Two weeks after inoculation, the formation of colonies was checked on each plant. Colonies were visible on susceptible plants, but resistant plants showed no symptoms. To map the resistance gene, linkage analysis was

conducted by using MAPMAKER/EXP 3.0 under the order command.

Results

Evaluation of powdery mildew resistance

AR 5 showed no symptoms (DI = 0, data not shown), whereas Harukei 3 was severely infected (DI = 4.1–4.9, data not shown). F_1 plants were moderately resistant to both pxA and pxB at both stages (DI = 0.5–1.8, data not shown), suggesting partial dominant inheritance of this trait. Average DI values of each RIL showed a continuous distribution from susceptible to resistant, suggesting oligogenic or polygenic control of this trait (Fig. 1), with a bias towards resistance. With inoculation of pxA, the distributions of RILs did not significantly differ ($P > 0.05$) between stages. However inoculation with pxB led to significantly different ($P < 0.01$) distributions, with more RILs showing complete resistance (mean DI = 0) at first-leaf stage ($n = 44$) than at cotyledon stage ($n = 16$). The distributions of RILs at the cotyledon stage differed significantly ($P < 0.01$), whereas those at first-leaf stage did not significantly differ ($P > 0.05$) between pxA and pxB.

Linkage map and map comparison

Among the 556 SSR primers tested, 171 (30.8%) were polymorphic between AR 5 and Harukei 3. Of 171 polymorphic markers, 2.9% deviated from the expected 1:1 segregation ratio at $P = 0.05$. The deviation was not huge, most ratios being below 2:1, and all polymorphic markers were used for linkage analysis. The constructed map spans 877 cM and consists of 167 markers, comprising 157 SSRs, 7 SCARs/CAPSs and 3 morphological markers segregating into 20 LGs (143 markers assigned at 5.0 LOD and 24 at 3.0 LOD; Fig. 2). Fourteen markers were unlinked or formed small linkage groups that could not be assigned to LGs. Thirty-six SSRs and six other markers were common between the AR5_H3 and PI_PS maps. The numbers of common markers in each LG of the AR5_H3 map ranged from 0 to 7. Seventeen LGs of the AR5_H3 map could be assigned to individual LGs of the PI_PS map. The marker orders were almost the same between maps, except for slight differences in LG VI and LG XII (markers indicated with asterisk in Fig. 2). No common marker was found in the remaining three LGs. To determine the corresponding LG, we selected one or two markers from each LG and mapped them on the PI_PS map by selective genotyping using the bin-mapping strategy. Two were assigned to LG V (named LG VB and LG VC) and one to LG IV (named LG IVA). Four SSR markers and one other marker were

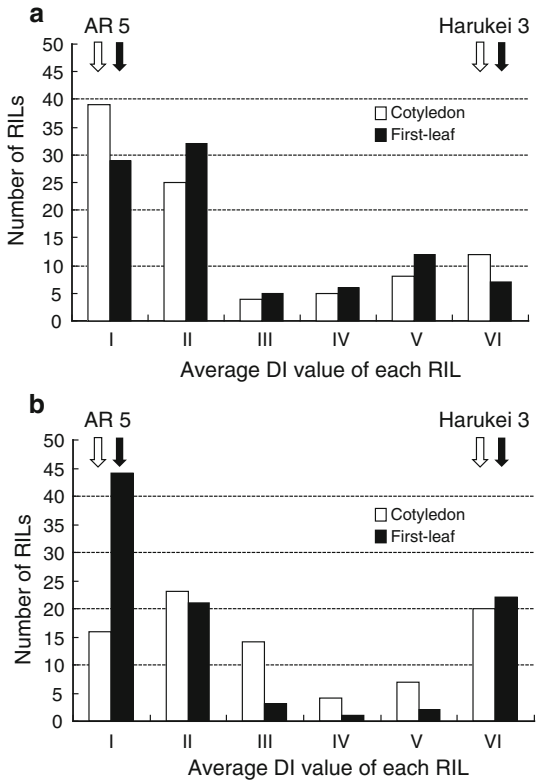
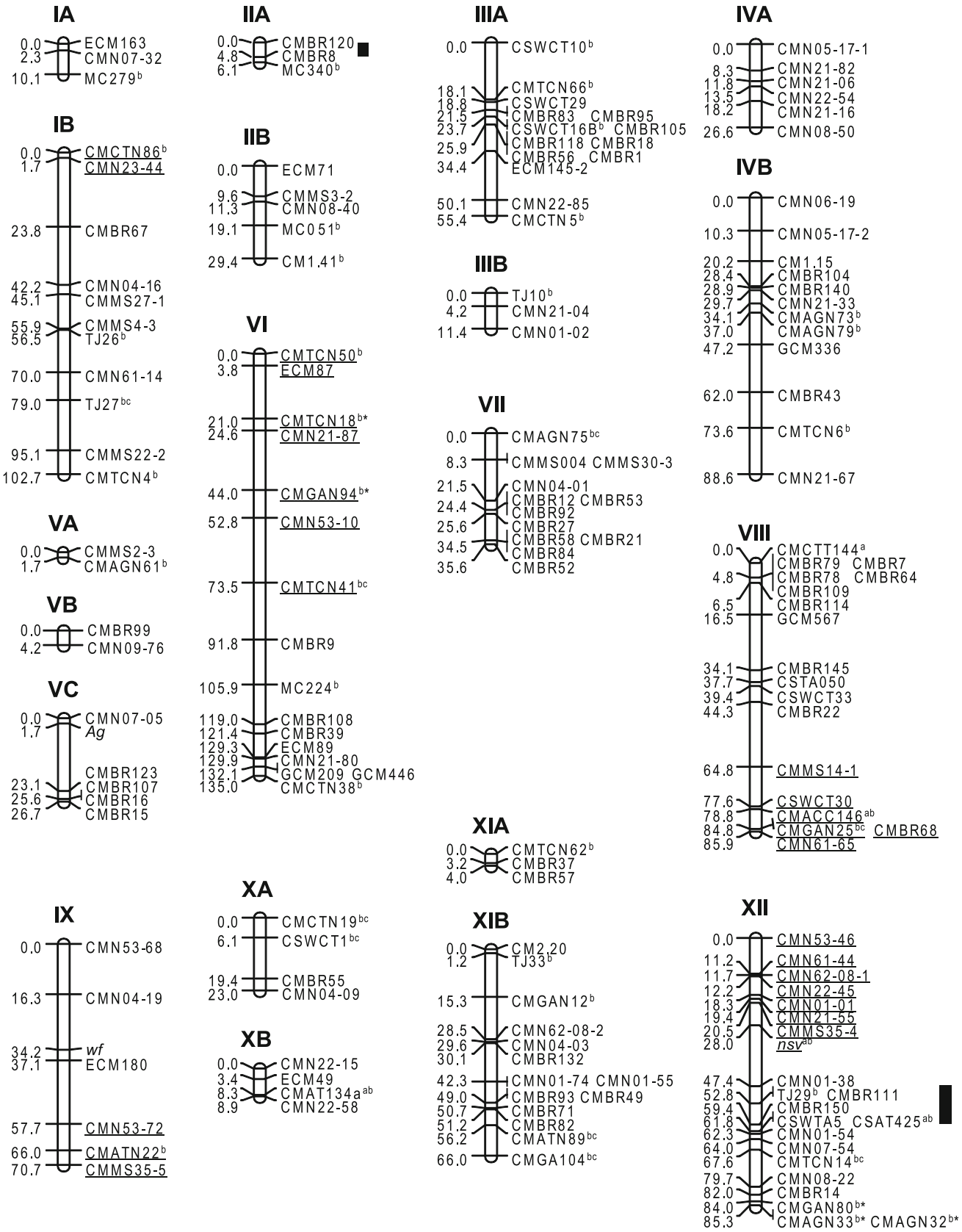


Fig. 1 Frequency distribution of mean disease index (DI) scores in recombinant inbred lines (RILs) derived from *Cucumis melo* AR 5× 'Harukei 3' under artificial infection by two strains (**a** pxA and **b** pxB) of *Podosphaera xanthii*. *I* DI = 0 (resistant), *II* 0 < DI ≤ 1, *III* 1 < DI ≤ 2, *IV* 2 < DI ≤ 3, *V* 3 < DI ≤ 4, *VI* 4 < DI ≤ 5 (susceptible)

common between the Ved_PI and AR5_H3 maps, and eight SSR markers were common between the US_TM and AR5_H3 maps. The locations of all of these shared markers in LGs were consistent with those on the three previous maps.

QTL analysis

The positions of LOD peaks, LOD scores, estimates of additive effects and the percentage of phenotypic variance explained (R^2) based on CIM for QTLs associated with resistance to the two powdery mildew isolates are summarized in Table 3. Regardless of strain (pxA or pxB) or plant stage (cotyledon or first leaf), two QTLs were detected, in LG IIA and LG XII. The R^2 values of the QTLs for resistance to pxA were similar at 22–28%. For resistance to pxB, the R^2 values of the QTL on LG XII (41–46%) were much larger than those of the QTL on LG IIA (12–13%). These results are comparable with those from the nonparametric Kruskal–Wallis rank-sum test (data not shown). The markers within the QTL confidence intervals determined by CIM always had significant *P* values by the Kruskal–Wallis rank-sum test. Moreover, the peaks identified by CIM were


IA
0.0 ECM163
2.3 CMN07-32
10.1 MC279b
IB
0.0 CMCTN86b
1.7 CMN23-44
23.8 CMBR67
42.2 CMN04-16
45.1 CMMS27-1
55.9 CMMS4-3
56.5 TJ26b
70.0 CMN61-14
79.0 TJ27bc
95.1 CMMS22-2
102.7 CMTCN4b
VA
0.0 CMMS2-3
1.7 CMAGN61b
VB
0.0 CMBR99
4.2 CMN09-76
VC
0.0 CMN07-05
1.7 Ag
23.1 CMBR123
25.6 CMBR107
26.7 CMBR16
CMBR15
IX
0.0 CMN53-68
16.3 CMN04-19
34.2 wf
37.1 ECM180
57.7 CMN53-72
66.0 CMATN22b
70.7 CMMS35-5
IIA
0.0 CMBR120
4.8 CMBR8
6.1 MC340b
IIB
0.0 ECM71
9.6 CMMS3-2
11.3 CMN08-40
19.1 MC051b
29.4 CM1.41b
VI
0.0 CMTCN50b
3.8 ECM87
21.0 CMTCN18b*
24.6 CMN21-87
44.0 CMGAN94b*
52.8 CMN53-10
73.5 CMTCN41bc
91.8 CMBR9
105.9 MC224b
119.0 CMBR108
121.4 CMBR39
129.3 ECM89
129.9 CMN21-80
132.1 GCM209 GCM446
135.0 CMCTN38b
XA
0.0 CMCTN19bc
6.1 CSWCT1bc
19.4 CMBR55
23.0 CMN04-09
XB
0.0 CMN22-15
3.4 ECM49
8.3 CMAT134aab
8.9 CMN22-58
IIIA
0.0 CSWCT10b
18.1 CMTCN66b
18.8 CSWCT29
21.5 CMBR83 CMBR95
23.7 CSWCT16Bb CMBR105
25.9 CMBR118 CMBR18
CMBR56 CMBR1
34.4 ECM145-2
50.1 CMN22-85
55.4 CMCTN5b
IIIB
0.0 TJ10b
4.2 CMN21-04
11.4 CMN01-02
VII
0.0 CMAGN75bc
8.3 CMMS004 CMMS30-3
21.5 CMN04-01
24.4 CMBR12 CMBR53
CMBR92
25.6 CMBR27
34.5 CMBR58 CMBR21
CMBR84
35.6 CMBR52
XIA
0.0 CMTCN62b
3.2 CMBR37
4.0 CMBR57
XIB
0.0 CM2.20
1.2 TJ33b
15.3 CMGAN12b
28.5 CMN62-08-2
29.6 CMN04-03
30.1 CMBR132
42.3 CMN01-74 CMN01-55
49.0 CMBR93 CMBR49
50.7 CMBR71
51.2 CMBR82
56.2 CMATN89bc
66.0 CMGA104bc
IVA
0.0 CMN05-17-1
8.3 CMN21-82
11.8 CMN21-06
13.5 CMN22-54
18.2 CMN21-16
26.6 CMN08-50
IVB
0.0 CMN06-19
10.3 CMN05-17-2
20.2 CM1.15
28.4 CMBR104
28.9 CMBR140
29.7 CMN21-33
34.1 CMAGN73b
37.0 CMAGN79b
47.2 GCM336
62.0 CMBR43
73.6 CMTCN6b
88.6 CMN21-67
VIII
0.0 CMCTT144a
CMBR79 CMBR7
4.8 CMBR78 CMBR64
CMBR109
6.5 CMBR114
16.5 GCM567
34.1 CMBR145
37.7 CSTA050
39.4 CSWCT33
44.3 CMBR22
64.8 CMMS14-1
77.6 CSWCT30
78.8 CMACC146ab
84.8 CMGAN25bc CMBR68
85.9 CMN61-65
XII
0.0 CMN53-46
11.2 CMN61-44
11.7 CMN62-08-1
12.2 CMN22-45
18.3 CMN01-01
19.4 CMN21-55
20.5 CMMS35-4
28.0 nsvab
47.4 CMN01-38
52.8 TJ29b CMBR111
59.4 CMBR150
61.8 CSWTA5 CSAT425ab
62.3 CMN01-54
64.0 CMN07-54
67.6 CMTCN14bc
79.7 CMN08-22
82.0 CMBR14
84.0 CMGAN80b*
85.3 CMAGN33b* CMAGN32b*

◀ **Fig. 2** Linkage map for AR 5× Harukei 3 recombinant inbred lines (RILs). The genotypes of 93 RILs were determined by using 157 SSR, 7 SCAR/CAPS, and 3 morphological markers. The *markers* are shown to the *right* of the linkage groups, and distances between markers are indicated in centimorgans to the *left*. *Markers* indicated with superscript *a*, *b*, and *c* are in common to the maps of Périn et al. (2002), Gonzalo et al. (2005) (the PI_PS map), and Zalapa et al. (2007), respectively. Linkage groups were aligned with the PI_PS map by using common SSR and SCAR markers, and linkage group nomenclature corresponds to that in Périn et al. (2002). The order of markers indicated with *asterisk* differs from that of the PI_PS map. *Underlined markers* were added to the linkage group by using LOD thresholds of 3.0, and all others were added at LOD thresholds of 5.0. QTLs involved in powdery mildew were located at the same position on two linkage groups (LG II and LG XII) regardless of strain or plant age, and they are presented as *bars* to the *right* of linkage groups

positioned at or near the position of the markers showing the highest *P* values by the Kruskal–Wallis rank-sum test (data not shown).

We classified the RILs according to the four possible genotypes of the markers closely linked to the QTLs on LG IIA and LG XII and calculated the mean DI values (Table 4). RILs with both QTLs were highly resistant to both strains at both stages, with DI = 0.1–0.4. RILs with one QTL were moderately resistant, with DI = 0.3–2.4. RILs with neither QTL were susceptible to both strains at both stages. These results confirm that both QTLs provide partial resistance, and both are necessary to high resistance, indicating that they act additively.

Validation of marker–resistance association in an independent population and mapping of powdery mildew resistance gene

The susceptible parent MSL031L-5-26 showed pxA colony growth after 2 weeks, confirming its susceptibility to powdery mildew, whereas the resistant parent M03-6 and F_1s showed no colonies. Two markers (CMBR8 and CMBR120) linked to the QTL on LG IIA and one marker (CMBR111) linked to another QTL on LG XII were used for screening of polymorphism between the parents.

Table 3 QTLs detected for resistant to two strains of powdery mildew (pxA, pxB) in melon recombinant inbred lines using composite interval mapping

Strain	Stage	Linkage group	Flanking markers	Position[a]	LOD	Additive effect[b]	R^{2c}
pxA	Cotyledon	LG IIA	CMBR8–CMBR120	4.0	12.6	–0.93	0.27
		LG XII	CMN01_38–TJ29	52.9	11.7	–0.87	0.23
	First-leaf	LG IIA	CMBR8–CMBR120	4.8	11.6	–0.83	0.28
		LG XII	CMN01_38–TJ29	51.5	8.9	–0.76	0.22
pxB	Cotyledon	LG IIA	CMBR8–CMBR120	2.0	5.1	–0.71	0.13
		LG XII	CMBR111–CMBR150	57.0	13.7	–1.27	0.41
	First-leaf	LG IIA	CMBR8–CMBR120	4.0	6.4	–0.72	0.12
		LG XII	CMBR111–CMBR150	55.0	17.9	–1.40	0.46

[a] Expressed in Kosambi cM

[b] Additive effects indicate an additive main effect of the parent contributing the higher-value allele: positive values indicated that higher-value alleles come from AR 5 and negative values indicate that higher-value alleles come from Harukei 3

[c] R^2 is the proportion of the phenotypic variance explained by the QTL

Table 4 Mean disease index (DI) at the cotyledon and first-leaf stages after inoculation of two strains of powdery mildew (pxA, pxB) and marker genotypes around two QTLs in recombinant inbred lines (RILs)

Marker genotype[a]		No. of RILs[b]	Mean DI[c]			
QTL on LG IIA CMBR8 and CMBR120	QTL on LG XII CMBR111		pxA		pxB	
			Cotyledon	First-leaf	Cotyledon	First-leaf
A	A	25	0.1a	0.1a	0.4a	0.1a
A	B	15	0.4ab	0.4ab	2.4bc	1.0b
B	A	23	0.6b	0.9b	1.3b	0.3a
B	B	21	3.9c	3.5c	4.2c	4.5c

Mean DIs followed by the same letter are not significantly different at the 5% level by the Mann–Whitney *U*-test with Bonferroni's correction

[a] Marker genotype designation: *A* homozygous AR 5; *B* homozygous Harukei 3

[b] Recombinant and heterozygous genotypes were excluded from the analysis

[c] Significant difference among the four genotypes by Kruskal–Wallis analysis by the 1% level

Among them, CMBR8 and CMBR120, on LG IIA, were polymorphic: MSL031L-5-26 showed the same allele as Harukei 3 and M03-6 showed the same allele as AR 5 for both markers. Whereas, CMBR111, on LG XII, was not polymorphic between parents, and therefore M03-6 was inferred to possess one of the two resistance genes, which was located on LG IIA. To assess whether the markers linked to the resistance detected in the AR5_H3 RIL population could be useful in an independent population, we used CMBR8 and CMBR120 linked to the QTL on LG IIA to test the association with disease resistance in the F_2 population derived from MSL031L-5–206× M03-6. The segregation of response to powdery mildew in the F_2 population was 124 resistant : 55 susceptible. This agrees with the expected 3:1 segregation ($\chi^2 = 3.13$, $P = 0.08$). The relationship between marker genotype and powdery mildew resistance is shown in Table 5. Twenty-nine plants were homozygous for the alleles of the resistant parent, and all were resistant. Forty-eight plants were homozygous for the alleles of the susceptible parent, and all were susceptible. Linkage analysis revealed that the QTL on LG IIA was located between CMBR8 and CMBR120, at a distance of 3 cM from both markers.

Discussion

Linkage map

Among 556 SSR primers tested, 171 (30.8%) were polymorphic between AR 5 and Harukei 3. This polymorphism level was lower than that found between the parents used to derive the PI_PS map (49.6%) (Gonzalo et al. 2005). The parents used to derive the PI_PS map were PI 161375, in var. *conomon*, and 'Piel de Sapo', in var. *inodorus*. In contrast, AR 5 and Harukei 3 both belong to var. *cantalupensis*. The lower polymorphism between AR 5 and Harukei 3 can be attributed to their more similar genetic background.

Several molecular marker linkage maps from melon have been reported, with a large proportion of dominant markers (Baudracco-Arnas and Pitrat 1996, Wang et al. 1997). Several attempts at map comparison using codominant markers as anchor points have been made (Danin-Poleg et al. 2000; Oliver et al. 2001; Périn et al. 2002; Silberstein et al. 2003; Gonzalo et al. 2005; Zalapa et al. 2007). Among those maps, the PI_PS map can be used for map comparison because it was constructed exclusively with codominant and transferable markers such as RFLP, SSR and SNP markers. We constructed our AR5_H3 map using 157 SSRs, 7 SCARs/CAPSs and 3 phenotypic markers. It has 43 markers (37 SSRs and 6 others) in common with the previous maps, and 20 LGs could be aligned with LGs of the PI_PS map. The orders and distances of common markers were similar on both maps. In addition, the AR5_H3 map has five and eight common markers with the Ved_PI and US_TM map, respectively. Gonzalo et al. (2005) proposed that SSRs could be used as bridges to other maps to build a melon consensus maps. Our results confirm their conclusion and add more public SSRs to the linkage map. Our linkage map, with many codominant markers, could be useful for comparing melon maps derived from different populations.

Powdery mildew resistance

The reaction at first-leaf stage indicated that strain pxA is equivalent to race N1 (Hosoya et al. 2000) and pxB to race 1 (Bardin et al. 1999). However, line MR 1 was moderately resistant to pxA, whereas it is known to be fully resistant to N1 (M. Kuzuya, personal communication), suggesting different virulence between N1 and pxA. Strains belonging to the same race may differ in pathogenicity (Orihara et al. 2001), probably because of intra-race genetic variability. Recently, the appearance of new races of *P. xanthii* has been reported (Hosoya et al. 2000, McCreight et al. 2005). The variability of virulence of this fungus might make race identification complex and difficult. For clear definition of races, identification of resistance genes and clarification of the interaction between each gene and each race is necessary. An international standard differential set of rice lines that are monogenic for 24 kinds of resistance genes has been widely used to test rice blast resistance (Kobayashi et al. 2007). Differential melon lines have been selected on the basis of differences in resistance reaction. The development of differential cultivars which possess each resistance gene and the establishment of an international standard inoculation method can be expected to clarify the pathogen-

Table 5 Relationship between genotypes of markers linked to QTL and powdery mildew resistance in 179 F_2 plants

Marker genotype[a]		Powdery mildew resistance	
CMBR8	CMBR120	Resistant	Susceptible
A	A	29	0
H	H	81	0
B	B	0	48
A	H	5	0
H	A	4	0
H	B	1	4
B	A	1	1
B	H	3	2

I DI = 0 (resistant), *II* 0 < DI ≤ 1, *III* 1 < DI ≤ 2, *IV* 2 < DI ≤ 3, *V* 3 < DI ≤ 4, *VI* 4 < DI ≤ 5 (susceptible)

[a] Marker genotype designation: *A* homozygous for AR 5 allele, *H* heterozygous, *B* homozygous for Harukei 3 allele

esis of powdery mildew races and the gene-race interactions.

Two independent QTLs for powdery mildew resistance were detected by using RILs derived from the crosses between AR 5 and Harukei 3. From a segregation analysis of the same RILs, we previously hypothesised that the resistance of AR 5 to race 1 is controlled by two dominant genes, both of which are necessary for complete resistance (Fukino et al. 2004). The new results confirm that two loci are necessary to confer resistance to this fungus. Previously, three genes and two QTLs conferring powdery mildew resistance have been mapped in melon: *Pm-w* from WMR 29 on LG V, *Pm-x* from PI 414723 on LG II, *Pm-y* from VA 435 on LG XII (Pitrat 1991, Périn et al. 2002), *PmV.1* on LG V and *PmXII.1* on LG XII from PI 124112 (Perchepied et al. 2005). One QTL mapped on LG II might be the same locus as or linked to *Pm-x.* Another QTL on LG XII might be the same locus or linked to *Pm-y* or *PmXII.1*. Furthermore, a QTL for resistance to *Pseudoperonospora cubensis* (*pcII.1*) was identified near *Pm-x*. A cluster of resistance genes were inferred on LG V in melon (Perchepied et al. 2005); LG II might have a similar cluster. *Pm-w*, on LG V, should not have any relation to powdery mildew resistance of AR 5. Currently, there is no publicly available marker tightly linked to any powdery mildew resistance locus. We located one QTL between two SSR markers (CMBR8 and CMBR120) and the other very close to three SSR markers (TJ29, CMBR111and CMBR150).

The effect of the QTLs varied depending on the strain of *P. xanthii* and the stage of the plants; both QTLs had a similar effect on pxA, whereas the QTL on LG XII had a greater effect on pxB than the one on LG IIA. We inoculated 48 commercial F_1 cultivars with pxA and pxB at the cotyledon and first-leaf stage (data not shown). Most were susceptible to pxA at both stages and to pxB at the cotyledon stage, although they were resistant to pxB at first leaf. Similarly, when we inoculated the RILs with pxB, more RILs were resistant to pxB at first leaf than at cotyledon stage. These data suggest that specific combinations of fungal strain and resistance genes lead to different levels of resistance at different stages of growth. Different levels of powdery mildew resistance or different genes for resistance depending on leaf position have been reported in melon (Cohen 1993; McCreight 2003; Boiteux et al. 1995). In addition, environmental conditions contribute largely to the development of this fungus (Cohen et al. 2002). Further studies will be necessary to clarify the interaction among powdery mildew strains, plant stage, environmental condition and resistance genes.

We detected two QTLs by using RILs by leaf disc assays under a controlled environment. Whereas, resistance in an F_2 population derived from the cross between a susceptible line (MSL031L-5-26) and a resistant line (M03-6) with one QTL on LG IIA segregated as a single dominant trait by whole-plant assay, in which the resistant parent and F_1 progeny showed complete resistance. With inoculation of pxA, the average DI value of RILs which had one QTL on LG IIA was 0.4, the disease symptoms being very slight in those lines. It was assumed that in F_2 assay slight symptoms did not appear visibly, and individuals with one QTL were judged as complete resistance. The result indicated that when lines are selected under some conditions (e.g., those for the test of the F_2 population), the resistance gene on LG IIA is sufficient for complete resistance, and individuals with one resistance gene cannot be distinguished from those with two. In such cases, pyramiding of resistance genes would be impossible based on phenotype alone. Instead, the use of SSR markers linked to the two QTLs in MAS will allow the breeding of lines highly resistant to powdery mildew.

In the present study, QTLs for powdery mildew resistance was detected by the assays under a controlled environment. To test powdery mildew resistance using a uniform strain of the fungus under a natural condition will be almost impossible because of the contamination by naturally occurring strains. Resistance to naturally occurring strains of powdery mildew was evaluated using RILs in a plastic greenhouse at fruit set over three seasons with 78–93 RILs each year. The degree of disease symptom was scored by eye on a scale of 0 (no symptom) to 5 (severely infected) as a DI, and averaged over three seasons. RILs were classified according to the four genotypes the same as listed in Table 4. The mean DI values of the four groups were AA < AB < BA < BB. Thus, the effectiveness of pyramiding resistance genes and the applicability of the markers developed in the present study under a field environment as well as a controlled environment were indicated.

Several mechanisms of powdery mildew resistance in melon have been proposed. Kuzuya et al. (2000) reported different resistant reactions of PMR 5 to four races of powdery mildew: resistance to races 1 and N1 was related to a delay in fungal development, while resistance to races 2 and N2 was due to a reduction in the number of conidia. Cohen and Eyal (1988) reported that the rapid collapse of epidermal cells in resistant melon plants invaded by *P. xanthii* is accompanied by the accumulation of callose-like deposits and lignification. Rivera et al. (2002) found an earlier induction of β-1,3-glucanase transcripts in a resistant cultivar than in a susceptible one. However, the cited studies were focused on fungus–plant interactions; to clarify the mechanisms of powdery mildew resistance, fungus–gene interactions remains to be elucidated. These markers identified in the present study will be useful not only for MAS for resistance to powdery mildew, but also for identification of resistance genes to clarify the fungus-gene interactions, and

for map-based cloning of genes involved in resistance to elucidate the mechanisms of powdery mildew resistance.

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